IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:

Applicant	: Bouffard Fita, Fernando	
Appln. No.	: 10/562,392	Confirmation No: 8090
Filed	: December 19, 2005	Group Art Unit: 1627
Title	: ANAESTHETIC COMPOSITION FOR TOPICAL ADMINISTRATION COMPRISING LIDOCAINE, PRILOCAINE AND TETRACAINE	Examiner: Jody Lynn Karol

DECLARATION UNDER 37 CFR § 1.132
DECLARATION OF FERNANDO BOUFFARD FITA

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

To the Commissioner:

I, **Fernando Bouffard Fita**, do declare that:

1. I am a citizen of Spain and reside in Barcelona, Spain.

2. Based on my education and experience, I am considered to be one of ordinary skill in the art of making and testing anesthetic compositions, such as those currently claimed in the above-identified application. I have been collaborating with the R&D Department of APR S.A. since 2008 in the design and development of topical anesthetics. I have developed, implemented and scaled up several preparation processes related to anesthetic compositions such as that presently claimed. I obtained my Ph. D in Pharmacy in 1991 from the University of Barcelona. I directly managed the preparation, execution and evaluation of the two experiments presented in the Annexes cited in this declaration, which is relevant to my experience relating to the technology claimed and disclosed in the above application. My current CV is attached as Exhibit A.

3. I am named inventor of the claimed invention as disclosed in this patent application. I have reviewed and understand the above-identified application, the pending claims, the prior Office Actions, and the state of

the art references cited by the Examiner, e.g., Cassel (US 2002/0128285, hereinafter "Cassel"); Samuels et al. (US 2002/0006435 A1, hereinafter "Samuels"); Lutz et al. (US 5,750,139, hereinafter "Lutz"); and Santana et al. (US 2003/0103955 A1, hereinafter "Santana"). I am making the following statements as relating to what one of ordinary skill in the art would be taught or suggested by these cited references, alone or in combination, in relation to the pending claims of this application.

4. Therefore, being completely familiar with the subject matter of the present application, the cited references, and the properties of the compounds in the patent application and in the references, it is my opinion that, on comparison of the cited reference compounds with those potentially encompassed by the pending claims in the present application, the claimed triple anesthetic composition comprising lidocaine, prilocaine and tetracaine, is not taught or suggested to one of ordinary skill in the relevant arts by any one or a properly cited combination of the cited references in the prior Office Actions.

5. In particular, the data in the present specification and as presented in this Declaration establish that the combination of the three anesthetics (lidocaine, priolocaine, and tetracaine) for a topical anesthetic show surprising, unexpected and/or synergistic properties that cannot be attributable to any additive or separate properties of each or any combination of any two of these anesthetics.

6. The data in the present specification establish that these surprising, unexpected and/or synergistic properties of the combination of lidocaine, prilocaine and tetracaine, include, inter alia,
   (i) surprising, unexpected and/or synergistic enhanced anesthetic effect;
   (ii) surprising, unexpected and/or synergistic accelerated anesthetic activity; and/or
   (iii) surprising, unexpected and/or synergistic lower adverse effects.

7. Regarding surprising, unexpected and/or synergistic (a) enhanced anesthetic effect; (b) surprising, unexpected and/or synergistic accelerated anesthetic activity; and (c) lower adverse effects, paragraphs [0032] – [0037] of the present specification as originally filed summarize a very large clinical study involving 2700 patients ranging in age from 15-65 years of age (see, e.g., [0032]).

8. Paragraphs [0033] to [0036], and Tables 1, 2 and 3, of the present specification establish that the three anesthetic combination of lidocaine, prilocaine, and tetracaine showed surprising, unexpected and/or synergistic effects as compared to the combination of prilocaine and lidocaine alone.

9. These surprising, unexpected and/or synergistic effects included (a) enhanced anesthetic effect (as shown, e.g., in Table 1); (b) accelerated anesthetic effect (as shown, e.g., in Table 1); (c) lower adverse effects (as shown, e.g., in Tables 2 and 3).

10. Thus, the claimed triple anesthetic composition comprising lidocaine, prilocaine and tetracaine, shows surprising, unexpected and/or synergistic effects as compared to the cited art, and is not taught or suggested to one of ordinary skill in the relevant arts by any one or a properly cited combination of the cited references in the prior Office Actions.

(i) Additionally, the data as presented in this Declaration establish that these surprising, unexpected and/or synergistic properties of the combination of lidocaine, prilocaine and tetracaine, further include, inter alia, surprising, unexpected and/or synergistic greater stability; and/or surprising, unexpected and/or synergistic lower toxicity.

11. Regarding the surprising, unexpected and/or synergistic greater stability, the following comparative experiments were conducted and analyzed as follows.

12. To show that the presently claimed three anesthetic composition provided surprising unexpected and/or synergistic greater stability, the presently claimed three anesthetic topical composition was compared with single and double anesthetic combinations.

13. In particular, the stability of different combinations of Prilocaine, Lidocaine, and Tetracaine were evaluated by APR laboratory (Applied Pharma Research S.A.) from March 2008 to March 2009 under my guidance and supervision. The following compositions were evaluated, including the use of appropriate standards and controls. The compositions included the following:

(i) Batch LCOX/70: TERNARY COMPOSITION LIDOCAINE–PRILOCAINE–TETRACAINE ("LPT");
(ii) Batch LCOX/67: TETRACAINE ONLY ("T");
(iii) Batch LCOIX/71: BINARY COMPOSITION LIDOCAINE-TETRACAINE ("LT"); and
(iv) Batch LCOIX/74: BINARY COMPOSITION PRILOCAINE–TETRACAINE ("PT").

14. The SCHEDULE OF STABILITY CONTROLS (Time and Temperature), included the following:

	5°C	25°C	30°C	40°C
1 month (T=0)		T/LPT		T/LPT
6 month (T=6)	T/LPT/ LT/PT	T/LPT/ LT/PT	T/LPT	T/LPT/ LT/PT
12 month (T=12)		T/LPT		

15. The DATA EVALUATED included: (i) Assay and purity of Lidocaine, Prilocaine and Tetracaine; and (ii) known impurities; and unknown impurities.

16. The analytical methods for assay and purity used for assaying Lidocaine, Prilocaine, Tetracaine, and related impurities at 1 month interval included were the following:

(i) M44-07: HPLC Method for Lidocaine, Prilocaine, Tetracaine Assay; and M01-08: HPLC Method for Prilocaine and Lidocaine Purity. With this HPLC method it is possible the identification and the assay of o-toluidine (EP monograph for Prilocaine) and of 2,6–dimethylaniline (EP monograph for Lidocaine). For Prilocaine and Lidocaine were selected the above mentioned impurities because they are the same mentioned in USP 31 NF 26 monography of Lidocaine and Prilocaine cream;
(ii) M03-08: HPLC Method for Purity of Tetracaine. With this HPLC method it is possible the identification and the assay of 4-butylaminobenzoic acid (US 31 NF 26 monograph for Tetracaine);
(iii) The above mentioned HPLC methods are supported by "limited Validation" as follows: V01/08: Limited validation for Assay of Lidocaine, Prilocaine, Tetracaine; V02/08: Limited HPLC Validation for Purity of Tetracaine (4-butylaminobenzoic acid); and V03/08: Limited HPLC Validation Purity of Prilocaine and Lidocaine (o-toluidine, 2,6-dimethylaniline).

17. Then APR worked to improve analytical methods for assay Lidocaine, Prilocaine, Tetracaine and Tetracaine purity. (Method for Lidocaine and Prilocaine purity was already finalized and so no need to revise). Further investigation on analytical methods for assaying Lidocaine, Prilocaine, Tetracaine and Tetracaine purity and revised methods and validations were then used in order to provide better analytical conditions and to reduce the variability of the assay values obtained, although the variability (CV) of first issue analytical methods (M44-07 and M03-08) was satisfactory.

18. To find out the better analytical conditions for assaying Lidocaine, Prilocaine, Tetracaine and Tetracaine purity, APR tested different

solvent for diluting the analytical sample till find **Acetonitrile** as the best dilution agent. The resulting revisions to these methods include: (a) M44-07 Revision 1: HPLC Method for Lidocaine, Prilocaine Tetracaine Assay; and (b) M03-08 Revision 1: HPLC Method for Purity of Tetracaine. Acetonitrile was discovered to be a better dilution agent than water because it is independent from pH and does not influence the pH of final solution.

19. The acceptable range of pH for water used as dilution agent in method M44-07, M03-08 was from 5 to 7 in compliance to European Pharmacopoeia; hence the variability in water pH may influence the variability of analytical solution final pH and this gave a high variability (CV) during assay analysis of Lidocaine, Prilocaine and or Tetracaine with M44-07.

20. Using Acetonitrile as solvent in method for Tetracaine purity may ensure a better and more controlled analysis of 4-Butylaminobenzoic acid formation. Based on these results, APR performed a revision of limited validations for assay and purity methods, including: (i) V01/08 Revision 1: Limited HPLC validation for Assay of Lidocaine, Prilocaine, Tetracaine; and (ii) V02/08 Revision 1: Limited HPLC validation for Purity of Tetracaine (4-butylaminobenzoic acid).

21. In order to ensure continuity in the analytical results and stability evaluation, stability was tested from the third to the sixth month with both methods; and from the sixth month on, the analysis was performed only with revised method.

22. **PREPARATION AND STABILITY RESULTS OF FINAL FORMULATIONS FOR STABILITY EVALUATIONS**

Batch LCOX/70: TERNARY COMPOSITION LIDOCAINE–PRILOCAINE–TETRACAINE (LPT)

Tetracaine..... 4%

Lidocaine.....1.5%

Prilocaine.....1.5%

Parameter	**Method**	**Value**
Prilocaine – Identification	M44-07	Conform
Lidocaine –Identification	M44-07	Conform
Tetracaine - Identification	M44-07	Conform
Prilocaine Assay (%)	M44-07	100.86
Lidocaine Assay (%)	M44-07	101.50
Tetracaine Assay (%)	M44-07	104.21
Related substances / impurities		

o-Toluidine (w/w) (%)	M01-08	Absent
2,6-dimethylaniline (w/w) (%)	M01-08	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.025
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$= absent $RRt_{1.38}$= 0.048

Batch LCOX/67: TETRACAINE ONLY (T)

Tetracaine……4%

Parameter	**Method**	**Value**
Tetracaine - Identification	M44-07	Conform
Tetracaine Assay (%)	M44-07	103.24
Related substances / impurities		
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.320
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$= absent $RRt_{1.38}$= 0.104

Batch LCOIX/71: BINARY COMPOSITION LIDOCAINE-TETRACAINE (LT)

Tetracaine…..4%

Lidocaine…..1.5%

Parameter	**Method**	**Value**
Lidocaine –Identification	M44-07	Conform
Tetracaine - Identification	M44-07	Conform
Lidocaine Assay (%)	M44-07	102.40
Tetracaine Assay (%)	M44-07	104.96
Related substances / impurities		
2,6-Dimethylaniline (w/w) (%)	M01-08	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.034
Each Individual Unknown (by area) (%)	M01-08	$RRt_{1.38}$= 0.056

Batch LCOIX/74: BINARY COMPOSITION PRILOCAINE–TETRACAINE (PT)

Tetracaine…….4%

Prilocaine…..1.5%

Parameter	**Method**	**Value**
Prilocaine – Identification	M44-07	Conform
Tetracaine - Identification	M44-07	Conform
Prilocaine Assay (%)	M44-07	102.28
Tetracaine Assay (%)	M44-07	104.70

Related substances / impurities		
o-Toluidine (w/w) (%)	M01-08	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.029
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$= absent $RRt_{1.38}$= 0.063

The following results were obtained:

STABILITY 5 °C

LCOX/70: TERNARY COMPOSITION LPT

Parameter	Method	T_{6m}
Lidocaine Assay (%)	M44-07	100.4
Lidocaine Assay (%)	M44-07 rev 1	99.56
Prilocaine Assay (%)	M44-07	101.48
Prilocaine Assay (%)	M44-07 rev 1	99.72
Tetracaine Assay (%)	M44-07	117.21
Tetracaine Assay (%)	M44-07 rev 1	129.44
o-Toluidine (w/w) (%)	M01-08	Absent
2,6-Dimethylaniline (w/w) (%)	M01-08	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.291
4-Butylaminobenzoic acid (w/w) (%)	M03-08 Rev 1	0.027
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$=absent $RRt_{1.38}$= 0.074

LCOX/67: TETRACAINE ONLY (T)

Parameter	Method	T_{6m}
Tetracaine Assay (%)	M44-07	117.21
Tetracaine Assay (%)	M44-07 rev 1	129.44
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.291
4-Butylaminobenzoic acid (w/w) (%)	M03-08 Rev 1	0.027
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$=absent $RRt_{1.38}$= 0.074

LCOIX/71: BINARY COMPOSITION LT

Parameter	Method	T_{6m}
Lidocaine Assay (%)	M44-07	91.88
Lidocaine Assay (%)	M44-07 rev 1	Na
Tetracaine Assay (%)	M44-07	106.58

Tetracaine Assay (%)	M44-07 rev 1	Na
2,6-Dimethylaniline (w/w) (%)	M01-08	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.263
4-Butylaminobenzoic acid (w/w) (%)	M03-08 Rev 1	Na
Each Individual Unknown (by area) (%)	M01-08	$RRt_{1.38}$= 0.114

LCOIX/74: BINARY COMPOSITION PT

Parameter	**Method**	T_{6m}
Prilocaine Assay (%)	M44-07	103.43
Prilocaine Assay (%)	M44-07 rev 1	Na
Tetracaine Assay (%)	M44-07	104.29
Tetracaine Assay (%)	M44-07 rev 1	Na
o-Toluidine (w/w) (%)	M01-08	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.266
4-Butylaminobenzoic acid (w/w) (%)	M03-08 Rev 1	Na
Each Individual Unknown (by area) (%)	M01 -08	$RRt_{0.64}$=0.093 $RRt_{1.38}$= absent

STABILITY 25°C

LCOX/70: TERNARY COMPOSITION LPT

Parameter	**Method**	T_{1m}	T_{6m}	T_{12m}
Lidocaine Assay (%)	M44-07	99.31	98.59	Nd
Lidocaine Assay (%)	M44-07 rev 1	Na	100.00	101.63
Prilocaine Assay (%)	M44-07	97.94	99.01	Nd
Prilocaine Assay (%)	M44-07 rev 1	Na	100.89	101.03
Tetracaine Assay (%)	M44-07	101.09	102.93	Nd
Tetracaine Assay (%)	M44-07 rev 1	Na	100.70	101.16
o-Toluidine (w/w) (%)	M01-08	Absent	Absent	Absent
2,6-dimethylaniline (w/w) (%)	M01-08	Absent	Absent	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.140	0.293	nd
4-Butylaminobenzoic acid (w/w) (%)	M03-08 rev 1	Na	0.130	0.296
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$=0.023 $RRt_{1.38}$=0.043	$RRt_{0.64}$=0.079 $RRt_{1.38}$=absent	$RRt_{0.64}$=0.173 $RRt_{1.38}$=0.017

LCOX/67: TETRACAINE ONLY (T)

Parameter	Method	T_{1m}	T_{6m}	T_{12m}
Tetracaine Assay (%)	M44-07	107.66	102.91	Nd
Tetracaine Assay (%)	M44-07 rev 1	Na	97.71	97.70
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.255	0.814	Na
4-Butylaminobenzoic acid (w/w) (%)	M03-08 rev 1	Na	0.278	0.651
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$=0.082 $RRt_{1.38}$=0.088	$RRt_{0.64}$=0.369 $RRt_{1.38}$=absent	$RRt_{0.64}$=0.802 $RRt_{1.38}$=0.051

LCOIX/71: BINARY COMPOSITION LT

Parameter	Method	T_{1m}	T_{6m}	T_{12m}
Lidocaine Assay (%)	M44-07	Na	91.80	Na
Lidocaine Assay (%)	M44-07 rev 1	Na	Na	Na
Tetracaine Assay (%)	M44-07	Na	101.36	Na
Tetracaine Assay (%)	M44-07 rev 1	Na	Na	Na
2,6-dimethylaniline (w/w) (%)	M01-08	Na	Absent	Na
4-Butylaminobenzoic acid (w/w) (%)	M03-08	Na	0.373	Na
4-Butylaminobenzoic acid (w/w) (%)	M03-08 rev 1	na	Na	Na
Each Individual Unknown (by area) (%)	M01-08	Na	$RRt_{0.64}$=0.139 $RRt_{1.38}$=0.114	Na

LCOIX/74: BINARY COMPOSITION PT

Parameter	Method	T_{1m}	T_{6m}	T_{12m}
Prilocaine Assay (%)	M44-07	Na	106.78	Na
Prilocaine Assay (%)	M44-07 rev 1	Na	Na	Na
Tetracaine Assay (%)	M44-07	Na	105.98	Na
Tetracaine Assay (%)	M44-07 rev 1	Na	Na	Na
o-Toluidine (w/w) (%)	M01-08	Na	Absent	Na
4-Butylaminobenzoic acid (w/w) (%)	M03-08	Na	0.417	Na
4-Butylaminobenzoic acid (w/w) (%)	M03-08 rev 1	na	Na	na
Each Individual Unknown (by area) (%)	M01-08	Na	$RRt_{0.64}$=0.220 $RRt_{1.38}$=absent	Na

STABILITY 30°C

LCOX/70: TERNARY COMPOSITION LPT

Parameter	Method	T_{6m}
Lidocaine Assay (%)	M44-07	103.01
Lidocaine Assay (%)	M44-07 rev 1	103.53
Prilocaine Assay (%)	M44-07	102.48

Prilocaine Assay (%)	M44-07 rev 1	102.95
Tetracaine Assay (%)	M44-07	103.73
Tetracaine Assay (%)	M44-07 rev 1	103.40
o-Toluidine (w/w) (%)	M01-08	Absent
2,6-dimethylaniline (w/w) (%)	M01-08	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.358
4-Butylaminobenzoic acid (w/w) (%)	M03-08 Rev 1	0.255
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$=0.143 $RRt_{1.38}$=absent

LCOX/67: TETRACAINE ONLY (T)

Parameter	**Method**	T_{6m}
Tetracaine Assay (%)	M44-07	101.26
Tetracaine Assay (%)	M44-07 rev 1	101.60
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.849
4-Butylaminobenzoic acid (w/w) (%)	M03-08 Rev 1	0.438
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$=0.624 $RRt_{1.38}$=absent

STABILITY 40°C

LCOX/70: TERNARY COMPOSITION LPT

Parameter	**Method**	T_{1m}	T_{6m}
Lidocaine Assay (%)	M44-07	99.99	100.51
Lidocaine Assay (%)	M44-07 rev 1	Na	102.80
Prilocaine Assay (%)	M44-07	98.52	100.13
Prilocaine Assay (%)	M44-07 rev 1	Na	101.84
Tetracaine Assay (%)	M44-07	101.58	100.84
Tetracaine Assay (%)	M44-07 rev 1	Na	101.49
o-Toluidine (w/w) (%)	M01-08	Absent	Absent
2,6-dimethylaniline (w/w) (%)	M01-08	Absent	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.197	0.491
4-Butylaminobenzoic acid (w/w) (%)	M03-08 Rev 1	Na	0.453
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$=0.065 $RRt_{1.38}$=0.043	$RRt_{0.64}$=0.198 $RRt_{1.38}$=absent

LCOX/67: TETRACAINE ONLY (T)

Parameter	**Method**	T_{1m}	T_{8m}
Tetracaine Assay (%)	M44-07	104.22	97.31
Tetracaine Assay (%)	M44-07 rev 1	Na	97.20
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.491	1.179

4-Butylaminobenzoic acid (w/w) (%)	M03-08 Rev 1	Na	0.981
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$=0.302 $RRt_{1.38}$=0.107	$RRt_{0.64}$=1.061 $RRt_{1.38}$=absent

LCOIX/71: BINARY COMPOSITION LT

Parameter	Method	T_{1m}	T_{6m}
Lidocaine Assay (%)	M44-07	Na	93.20
Lidocaine Assay (%)	M44-07 rev 1	Na	Na
Tetracaine Assay (%)	M44-07	Na	102.09
Tetracaine Assay (%)	M44-07 rev 1	Na	na
2,6- dimethylaniline (w/w) (%)	M01-08	Na	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	Na	0.838
4-Butylaminobenzoic acid (w/w) (%)	M03-08 Rev 1	Na	na
Each Individual Unknown (by area) (%)	M0 -08	Na	$RRt_{0.64}$= 0.579 $RRt_{1.38}$=0.138

LCOIX/74: BINARY COMPOSITION PT

Parameter	Method	T_{1m}	T_{6m}
Prilocaine Assay (%)	M44-07	98.52	104.27
Prilocaine Assay (%)	M44-07 rev 1	Na	Na
Tetracaine Assay (%)	M44-07	101.58	103.27
Tetracaine Assay (%)	M44-07 rev 1	Na	Na
o-Toluidine (w/w) (%)	M01-08	Absent	Absent
4-Butylaminobenzoic acid (w/w) (%)	M03-08	0.197	0.885
4-Butylaminobenzoic acid (w/w) (%)	M03-08 Rev 1	Na	na
Each Individual Unknown (by area) (%)	M01-08	$RRt_{0.64}$=0.065 $RRt_{1.38}$=0.043	$RRt_{0.64}$= 0.424 $RRt_{1.38}$=absent

23. **CONCLUSIONS:** To achieve the conclusions written below, we had to observe the following parameters:

1. o-toluidine concentration: is a product of the degradation of Prilocaine described in Prilocaine monograph in the European Pharmacopoeia.

2. 2,6–dimethylaniline concentration: is a product of the degradation of Lidocaine described in Lidocaine monograph in the European Pharmacopoeia.

3. 4-butylaminobenzoic acid concentration: is a product of the degradation of Tetracaine described in Tetracaine monograph in the US Pharmacopoeia.

4. Unknown impurity detected at RRt 0.64 because achieved values are close to the allowed limit (0.2%) during the time of the study.

5. Unknown impurity detected at RRt 1.38 because achieved values are close to the allowed limit (0.2%) during the time of the study.

6. The relative amount of L, P and T is maintained in all the formulations evaluated (the ternary formulation, binary formulations and tetracaine formulation): L 1.5%, P 1.5% and T 4%. Therefore the effect of an anesthetic on the other ones within a formulation can be compared over all the formulations.

24. Once the data obtained was revised and studied we could conclude the following:

25. **Stability at 5 °C**:

(i) If we could observe the LPT composition, impurities due to the presence of Lidocaine and Prilocaine are not detected, but impurities from Tetracaine (4-butylaminobenzoic acid) are detected. The impurity from Tetracaine is in the ternary composition in lower values than the values which we can find in the composition with T only.

(ii) We could also detect that the values for the unknown impurities are much lower in the ternary composition (LPT) if we compare it with the values detected for the same parameter in the composition with T only.

(iii) Finally, in both binary compositions (LT, PT) we cannot find impurities from degradation of Lidocaine and Prilocaine, but we can find impurity from Tetracaine. In this case, the value for 4-butylaminobenzoic acid is bigger than the value found in the Ternary composition and lower than the value detected in the composition of Tetracaine only.

(iv) Referring to the unknown impurities we can observe that the values in the binary compositions (PT, LT) are bigger than the ternary composition (LPT) but lower than the composition with Tetracaine only (T).

(v) So, we can conclude that at 5°C, and in stability terms, the better formulation is the Ternary composition (LPT).

26. Stability at 25 °C

(i) We can observe similar results as the ones achieved at 5°C.

(ii) The ternary composition (LPT) presents products from the Tetracaine degradation and unknown impurities at RRt 0.64 and 1.38.

(iii) These values are much bigger in the case of the composition with Tetracaine only during the time of the research at these temperature conditions.

(iv) For binary compositions (PT, LT) the values of the known and unknown impurities are similar. These values are bigger than the ternary composition and lower than the composition with T only.
(v) Then, at these temperature conditions, we can conclude that the ternary composition is the better from an stability point of view.

27. Stability at 30 °C
(i) The results in the ternary composition continue in the same line of the others temperature conditions.
(ii) The results are much bigger in the composition with T only.
(iii) We have no data for the binary compositions (LT, PT).

28. Stability at 40 °C: At this temperature, we control accelerated stability, so it is normal that at this conditions the product becomes unstable.
(i) Evaluating the results obtained in the binary compositions or in the product with Tetracaine only, it can be assumed that the instability at 40 °C is strictly due to Tetracaine.
(ii) The gel with Tetracaine only is less stable than the binary composition with Tetracaine and than the ternary blend, being the last one the best one in stability terms.

29. From a stability point of view and after analyzing the whole data obtained during this year we can conclude that Prilocaine and Lidocaine stabilize Tetracaine when they are in the same formulation.

30. The above results clearly demonstrate that the claimed triple anesthetic composition comprising lidocaine, prilocaine and tetracaine, shows surprising, unexpected and/or synergistic increased stability as compared to single or double anesthetic compositions, such as those suggested or taught by the cited state of the art. This is clearly shown by the surprising, unexpected and/or synergistic decreased presence of known and unknown impurities with the claimed triple anesthetic composition comprising lidocaine, prilocaine and tetracaine, as compared to single or double anesthetic compositions.

31. Thus, the claimed triple anesthetic composition comprising lidocaine, prilocaine and tetracaine, shows surprising, unexpected and/or synergistic effects as compared to the cited art, and is not taught or suggested to one of ordinary skill in the relevant arts by any one or a properly cited combination of the cited references in the prior Office Actions.

32. Additionally, the data as presented in this Declaration establish that these surprising, unexpected and/or synergistic properties of the combination of lidocaine, prilocaine and tetracaine, further include, inter alia,surprising, unexpected and/or synergistic lower toxicity.

33. Regarding the surprising, unexpected and/or synergistic lower toxicity, the following comparative experiments were conducted and analyzed as follows.

34. To show that the presently claimed three anesthetic composition provided surprising unexpected and/or synergistic lower toxicity, the presently claimed three anesthetic topical composition was compared with single and double anesthetic combinations.

35. In particular, the toxicity of different combinations of Prilocaine, Lidocaine, and Tetracaine were evaluated, including the use of appropriate standards and controls.

36. The objective of this research is to demonstrate the synergistic effect in the decreasing of the adverse effects when we use a combined anesthetic with Lidocaine (L), Prilocaine (P) and Tetracaine (T), compared with each ingredient alone and in the same total concentration.

(i) The following design parameters are taken into account: in order to have a valid comparison, the compositions have to maintain the same total amount of anesthetic in each composition which is going to be compared; and
(ii) Testing different ranges of concentration according to claims of the patent application.

37. The 24 anesthetic compositions to be tested are the following:

1) The lower concentration range which is claimed: 0.5L + 0.5P + 0.5T (1.1), compared with: 1.2) 1.5L; 1.3) 1.5P; and 1.4) 1.5T;

2) The higher concentration range which is claimed: 5L + 5P + 8T (2.1), compared with: 2.2) 18L; 2.3) 18P; and 2.4) 18T;

3) Composition 1.5L + 1.5P + 4T (3.1), compared with: 3.2) 7L; 3.3) 7P; and 3.4) 7T

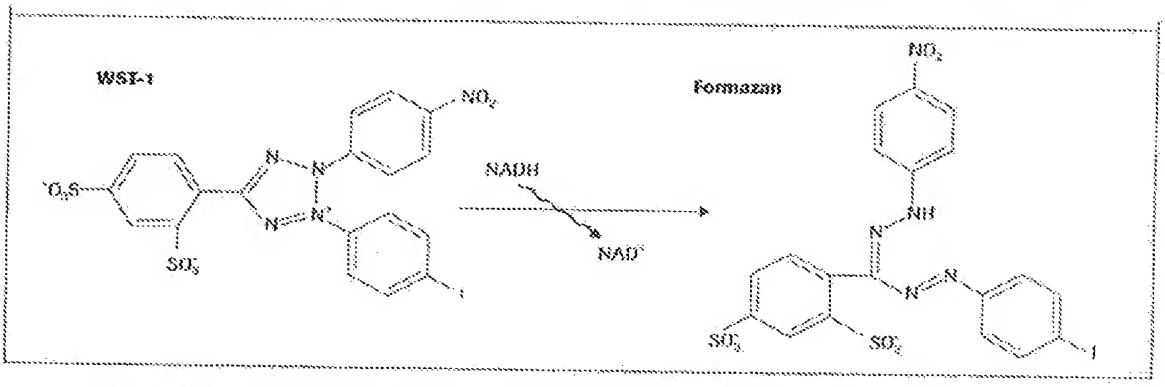
4) The presently claimed Ternary composition which keeps the same proportion of each agent as the above mentioned point (1.5L + 1.5P + 4T), but with a total sum of the anesthetic similar to EMLA and AMLI (5 parts of total anesthetic). In the same way two other ternary combinations will be tested which keep the total sum of anesthetic equal to EMLA and AMLI (5 parts): 4.1) 1.07L + 1.07P + 2.86T; 4.2) 1.5L + 1.5P + 2T; and 4.3) 1.5L + 2P + 1.5T,

compared with: 4.4) 5L 4.5) 5P; 4.6) 5T; 4.7) 2.5L + 2.5P (EMLA); and 4.8) 2.5L + 2.5T (AMLI).

5) Composition 1.5L + 1.5P + 8T (5.1) compared with: 5.1) 11L; 5.2) 11P; and 5.3) 11T.
(Concentrations of each anesthetic are in % w/w.)

38. MATERIALS AND METHODS: In order to compare the above mentioned we study the cytotoxicity of these 24 anesthetic compositions on CaCO-2 cells (human epithelial cells) by determining cell viability through WST-1. The study was carried out 24 hours after the treatment with the different anesthetic compositions, and four independent tests were done in triplicate.

39. The cytotoxic effect of a compound is determined by evaluating the percentage of cell death which the compound produces in comparison with a group of control cells which have not been treated. In order to do this, cell viability is measured by determining metabolic activity through a WST-1 (Roche) test. This method is based on the capacity of cells to obtain the energy necessary in order to continue their functions and to produce cell growth. For this reason, cells which are metabolically active (alive) reduce tetrazolium salts to formazan by means of the enzyme succinate-tetrazolium reductase (of the mitochondrial respiratory chain). The resulting formazan can be detected colourimetrically (see Figure 1).



In contrast, this reaction does not occur with damaged or dead cells.

Chemical reaction produced by the enzyme succinate-dehydrogenase of the mitochondrial cell chain. Formation of formazan from WST-1.

40. In the first instance, a preliminary test was carried out in order to determine the optimum working concentration which would allow differences to occur in the cytotoxicity of the different anesthetic compositions, as well to verify that the excipient used would not, itself,

produce toxicity. A first test was carried out in which the anesthetic compositions were diluted 1:10 in the growth medium. This caused the death of all the cells within a few hours after treatment. A second test was then carried out in which the anesthetic compositions were diluted 1:1000 in the growth medium. This provided appropriate results in order to be able to carry out a comparison between the anesthetic compositions.

41. Due to the viscosity of the anesthetic compositions, various tests were done in order to determine the procedure which would make reproducing the results the easiest. In the end, a dilution of 1:10 in cell growth medium was chosen. This was done 24 hours before the experiments were carried out in order to homogenize the dilution. At the time of the test, and after tempering the preparation, a second dilution of 1:100 in complete growth medium was carried out.

42. **RESULTS:** 24 anesthetic compositions were tested on CaCO-2 cells in a final optimum dilution of 1:1000 of the cell medium. Toxicity was analyzed by measuring metabolic cell activity in (untreated) control cells and cells treated with the anesthetic compositions.

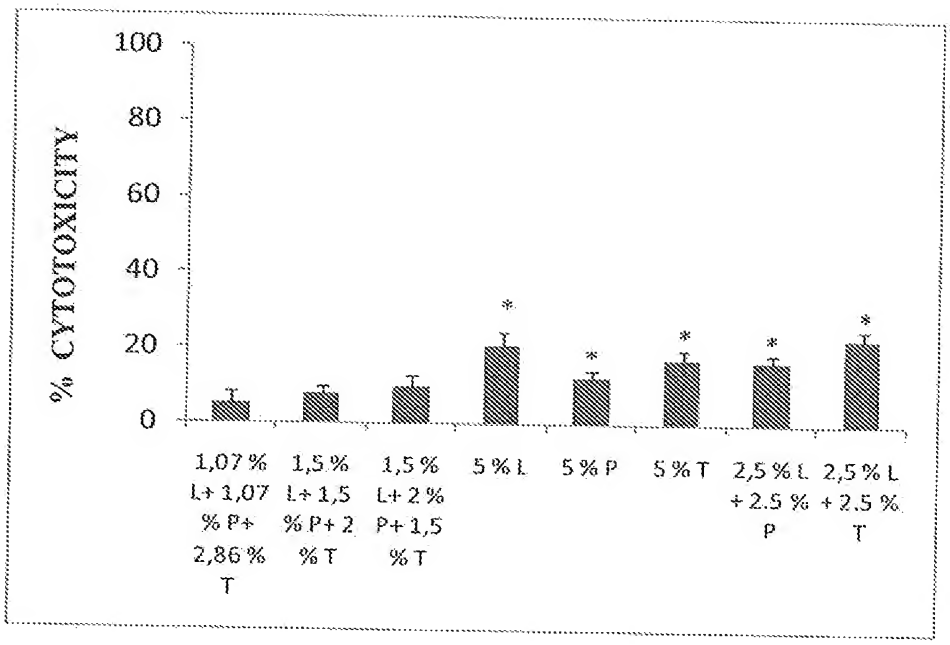
43. The table above shows the average percentage of toxicity caused by the anesthetic compositions on the CaCO-2 cells after 24 hours of treatment, compared to the (untreated) control cells. The averages given here are the averages of four independent tests carried out in triplicate. The standard deviation (SD) is given as well as the standard error of the mean (SEM). The p-value of the Student t-test is also given for each group of samples compared with the reference (REF) in each case. Differences are significant for $p<0.05$ (5.0E-02).

Composition	Samples	Medias	SD		SEM
EXCIPIENT		6	9	3	
0,5% L+0,5% P+0,5% T	1.1	2	8	2	REF.
1,5 % L	1.2	3	5	2	4,4E-01
1,5 % P	1.3	5	7	2	1,9E-01
1,5 % T	1.4	6	10	3	1,7E-01
5% L+ 5% P+ 8% T	2.1	23	16	5	REF.
18 % L	2.2	25	16	5	3,8E-01
18 % P	2.3	20	20	6	3,7E-01
18 % T	2.4	64	16	5	1,0E-06
1,5% L+1,5% P+4% T	3.1	42	17	5	REF.
7 % L	3.2	44	15	4	5,0E-01
7 % P	3.3	44	20	6	4,7E-01
7 % T	3.4	56	18	5	5,1E-02
1,07 % L + 1,07 % P+ 2,86 % T	4.1	5	10	3	REF.
1,5 % L+ 1,5 % P+ 2 % T	4.2	8	8	2	2,5E-01
1,5 % L+ 2 % P+ 1,5 % T	4.3	10	11	3	1,6E-01
5 % L	4.4	21	11	3	8,7E-04
5 % P	4.5	12	7	2	3,3E-02
5 % T	4.6	17	9	3	3,5E-03
2,5 % L + 2.5 % P	4.7	17	7	2	2,6E-03
2,5 % L + 2.5 % T	4.8	23	7	2	4,6E-05
1,5% L + 1,5% P + 8% T	5.1	22	13	4	REF.
11 % L	5.2	22	10	3	4,5E-01
11 % P	5.3	16	12	3	1,2E-01
11 % T	5.4	50	17	5	2,5E-04

44. **CONCLUSIONS:** A cytotoxicity study was carried out on 24 anesthetic compositions in a cell culture: CaCO-2. The results indicate, in the first instance, that the excipient (in the dilution used) displays no toxicity in the cell cultures. The results obtained, therefore, are due entirely to the effects of the different anesthetics. The result which stands out the most in the test carried out is the higher toxicity of the T anesthetic composition, while the L and P anesthetics presented lower toxicity, their results being quite similar.

45. In all cases the ternary combination of anesthetics (L,P,T) presents a lower toxic level than any of them separate. It is especially notable when we compare the ternary combination with T alone.

46. Below we can see the graphic representation of these results:



47. As we can see in the figure above, in all studied cases, the ternary anesthetic combination presents a lower toxicity level compared with the rest of the samples.

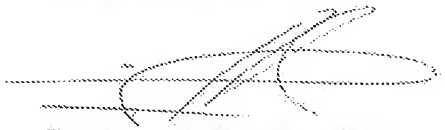
48. From this information one of ordinary skill in the art would conclude that a three anesthetic combination (L, P, T) presents a surprising, unexpected, and/or synergistic lower toxicity in human epithelial cells than the anesthetic EMLA and AMLI in the same total sum of anesthetic (5 parts).

49. Thus, the claimed triple anesthetic composition comprising lidocaine, prilocaine and tetracaine, shows surprising, unexpected and/or synergistic effects as compared to the cited art, and is not taught or suggested to one of ordinary skill in the relevant arts by any one or a properly cited combination of the cited references in the prior Office Actions.

50. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and any patent issuing thereon.

Dated: 1-12-2010

Respectfully submitted



Fernando Bouffard Fita

Enclosed:
Exhibit A: curriculum vitae of Fernando Bouffard Fita